TN5 TRANSPOSASE MUTANTS AND THE USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. provisional patent application Serial Number 60/463,470, filed on April 17, 2003, incorporated by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with United States government support awarded by the following agency: NIH, Grant No. GM50692 and USDA, Grant No. 02-CRHF-0-6055. The United States government has certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] Bacterial transposons such as Tn5 evolved within the cell by maintaining a low mobility level. While necessary for the transposon to survive, the low mobility level has inhibited the ability of researchers to detail the molecular transposition process and to exploit the transposition process for use, e.g., in the development of new diagnostic and therapeutic resources. Tn5 is a conservative "cut and paste" transposon of the IS4 family (Rezsohazy, R., Hallet, B., Delcour, J., and Mahillon, J., "The IS4 family of insertion sequences: evidence for a conserved transposase motif," Mol Microbiol. 9:1283-1295 (1993)) that encodes a 53 kD transposase protein (Tnp) that is responsible for its movement. The wild-type Tn5 transposase (Tnp) amino acid and nucleic acid sequences are known (Ahmed, A. and Podemski, L. The Revised Sequence of Tn5. Gene 154(1),129-130(1995), incorporated by reference as if set forth herein in its entirety). A nucleic acid sequence that encodes wild-type Tn5 Tnp is attached as SEQ ID NO:1. A polypeptide sequence encoded by SEQ ID NO:1 which corresponds to wild-type Tn5 Tnp is attached as SEQ ID NO:2.

[0004] The Tnp protein facilitates movement of the entire element by binding initially to each of two 19 bp specific binding sequences termed outside end (OE; SEQ ID NO:3), followed by formation of a nucleoprotein structure termed a synapse, blunt ended cleavage of each end, association with a target DNA, and then strand transfer (Reznikoff, W.S., Bhasin, A., Davies, D.R., Goryshin, I.Y., Mahnke, L.A., Naumann, T., Rayment, I., Steiniger-White, M., and Twining, S.S., "Tn5: A molecular window on transposition," Biochem. Biophys. Res. Commun. 266:729-34 (1999)). Tn5 Tnp can also promote movement of a single insertion sequence by using

a combination of OE and inside end (IE; SEQ ID NO:4) sequences. The IE is also 19 bp long and is identical to OE at 12 of 19 positions. *In vivo*, Tn5 Tnp exhibits a marked preference for OE in *E. coli*. Transposase recognition and binding to IE is inhibited in *E. coli* by the presence of four dam methylation sites (GATC palindromes) which add four methyl groups per inside end sequence (IE^{ME}; also depicted as SEQ ID NO:4, methylation not shown) (Yin, J.C.P., Krebs, M.P., and Reznikoff, W.S., "Effect of dam Methylation on Tn5 Transposition," J. Mol Biol., 199:35-45 (1988), incorporated by reference as if set forth herein in its entirety). This methylation reduces transposition by reducing protein-DNA primary recognition (Jilk, R.A., York, D., and Reznikoff, W.S., "The organization of the outside end of transposon Tn5," J. Bacteriol. 178:1671-1679 (1996)).

[0005] Tn5 transposon also encodes an inhibitor protein that can interfere with transposase activity. The inhibitor-encoding sequence overlaps with the sequence that encodes the transposase. An AUG in the wild-type Tn5 Tnp gene that encodes methionine at transposase amino acid 56 is the first codon of the inhibitor protein. Replacement of the methionine at position 56 with an alanine has no apparent effect upon the transposase activity. However, it prevents translation of the inhibitor protein and thus results in a higher transposition rate. Weigand, T. W. and W. S. Reznikoff, "Characterization of Two Hypertransposing Tn5 Mutants," J. Bact. 174:1229-1239 (1992), incorporated herein by reference.

[0006] A principal roadblock to understanding how Tn5 Tnp works is the fact that purified wild-type Tnp has no detectable activity *in vitro*. Recently, a double mutant hyperactive form of transposase ("Tnp EK/LP") that promotes the transposition reaction *in vitro* was developed (U.S. Patent No. 5,965,443, incorporated herein by reference in its entirety). The Tnp EK/LP protein differs from wild-type Tn5 Tnp at position 54 (Glu to Lys mutation) and at position 372 (Leu to Pro mutation), in addition to a non-essential but advantageous change at position 56 that prevents production of the inhibitor protein. The modified hyperactive Tnp protein increases the dramatic preference for OE termini of wild-type Tn5 Tnp. In addition, certain modifications on the OE sequence have been shown to increase the transposition frequency by Tnp EK/LP (U.S. Patent No. 5,925,545 and U.S. Patent No. 6,437,109, both of which are herein incorporated by reference in their entirety). Tnp EK/LP has clarified many aspects of Tn5 transposition that were not previously adequately addressable *in vivo*.

[0007] Another recent development in Tn5 research involves the identification of Tn5 mutants that have a higher avidity for IE than OE sequences (U.S. Patent No. 6,406,896, which is herein incorporated by reference in its entirety). These mutants contain a mutation at amino acid

position 58 and can further contain a mutation at amino acid position 8, 344, or both. Both unmethylated and methylated IE (IE^{ME}) sequences can be used efficiently for transposition by these Tn5 mutants.

[0008] *In vitro* polynucleotide transposition is a powerful tool for introducing random or targeted mutations into a genome. Useful *in vitro* transposition systems based upon the Tn5 transposon are disclosed in U.S. Patent No. 5,948,622, U.S. Patent No. 6,159,736 and U.S. Patent No. 6,406,896, all of which are incorporated herein by reference in their entirety.

BRIEF SUMMARY OF THE INVENTION

[0009] The present invention is summarized in that Tn5 Tnp mutants modified relative to the wild-type enzyme at amino acid positions 54, 242, and 372 have higher transposase activities than the wild-type enzyme. These mutants have greater avidity than the wild-type Tnp for at least one of a wild-type Tn5 outside end sequence as defined by SEQ ID NO:3 and a modified Tn5 outside end sequence as defined by SEQ ID NO:5 and can be used in a variety of *in vitro* and *in vivo* transposition applications.

[0010] In one aspect, the present invention relates to a polypeptide or isolated polypeptide that contains a Tn5 Tnp mutant of the present invention.

[0011] In another aspect, the present invention relates to a nucleic acid or isolated nucleic acid that contains a polynucleotide encoding a Tn5 Tnp mutant of the present invention. Optionally, the nucleic acid can contain a transcription control sequence operably linked to the Tnp mutant-encoding polynucleotide. A host cell containing the above nucleic acid is also within the scope of the present invention.

[0012] In another aspect, the present invention relates to a method of using a polypeptide containing a Tn5 Tnp mutant of the present invention for inter- or intra-molecular transposition *in vitro* as described in U.S. Patent No. 5,948,622.

[0013] In another aspect, the present invention relates to a method for forming a synaptic complex using a Tn5 Tnp mutant of the present invention and further introducing the complex into a target cell to make random or quasi-random insertional mutations in the cellular nucleic acid as described in U.S. Patent No. 6,159,736.

[0014] The invention will be more fully understood upon consideration of the following detailed description taken in conjunction with the accompanying drawing.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0015] Figs. 1A and 1B show agarose gel pictures of *in vitro* transposition reactions for Tn5 Tnp mutants EK54/LP372/PA242 and EK54/LP372, respectively, using pKJ1 as the substrate. All important substrate, intermediate, and product DNAs are labeled: sc = supercoiled, oc= open circle, Tn = transposon, dbb = donor backbone.

[0016] Fig. 1C shows a plot of the percentage of supercoiled substrate in each lane (determined by quatitation with Total Lab software) of Figs. 1A and 1B versus time. The data were fit to a one-phase exponential decay equation to determine the observed rate. The error bars represent one standard deviation.

DETAILED DESCRIPTION OF THE INVENTION

[0017] The term "polypeptide" and the term "protein" are used interchangeably in the specification and claims.

[0018] The term "isolated polypeptide" or "isolated nucleic acid" used in the specification and claims means a polypeptide or nucleic acid isolated from its natural environment or prepared using synthetic methods such as those known to one of ordinary skill in the art. Complete purification is not required in either case. Amino acid or nucleotide sequences that flank a polypeptide or nucleic acid in nature can but need not be absent from the isolated form. A polypeptide and nucleic acid of the invention can be isolated and purified from normally associated material in conventional ways such that in the purified preparation the polypeptide or nucleic acid is the predominant species in the preparation. At the very least, the degree of purification is such that the extraneous material in the preparation does not interfere with use of the polypeptide or nucleic acid of the invention in the manner disclosed herein. The polypeptide or nucleic acid is preferably at least about 85% pure, more preferably at least about 95% pure and most preferably at least about 99% pure.

[0019] Further, an isolated nucleic acid has a structure that is not identical to that of any naturally occurring nucleic acid or to that of any fragment of a naturally occurring genomic nucleic acid spanning more than three separate genes. The term therefore covers, for example, (a) a DNA that has the sequence of part of a naturally occurring genomic DNA molecule but which is not flanked by both of the coding sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner such that the resulting molecule is not identical to any

naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Specifically excluded from this definition are nucleic acids present in mixtures of (i) DNA molecules, (ii) transfected cells, and (iii) cell clones, e.g., as these occur in a DNA library such as a cDNA or genomic DNA library. An isolated nucleic acid molecule can be modified or unmodified DNA or RNA, whether fully or partially single-stranded or double-stranded or even triple-stranded. A modified nucleic acid molecule can be chemically or enzymatically induced and can include so-called non-standard bases such as inosine.

[0020] Tn5 Tnp mutants that differ from the wild-type enzyme at amino acid positions 54 and 372 (the 54/372 mutants) have previously been shown to possess higher than wild-type transposase activity, making these mutants suitable for *in vitro* transposition applications (U.S. Patent No. 5,965,443 and U.S. Patent No. 5,925,545). It is disclosed here that introducing another mutation to the 54/372 mutants at amino acid position 242 (the 54/242/372 mutants) will not abolish the higher-than-wild-type activity of the 54/372 mutants and in many cases will further increase the transposase activity of the 54/372 mutants. In addition, modifications on an OE sequence that are known to increase the transposition frequency of the 54/372 mutants (U.S. Patent No. 5,925,545 and U.S. Patent No. 6,437,109) can similarly increase the transposition frequency of the 54/242/372 mutants. Accordingly, a 54/242/372 mutant can be used similarly as a 54/372 mutant in various *in vitro* transposition applications as described in U.S. Patent No. 5,925,545, U.S. Patent No. 5,948,622, and U.S. Patent No. 6,159,736.

[0021] As shown in Example 2 provided below, when the modified OE sequence defined by SEQ ID NO:5 is employed for *in vitro* transposition, all eight 54/242/372 mutants constructed by the inventors as examples of the present invention displayed higher transposition activity than the 54/372 mutant. When wild-type OE sequence defined by SEQ ID NO:3 is employed, five out of eight 54/242/372 mutants displayed higher *in vitro* transposition activity than the 54/372 mutant. Although three of the eight 54/242/372 mutants catalyzed DNA transposition at a lower rate using the wild-type OE sequence than the 54/372 mutant, they nonetheless displayed *in vitro* transposition activity and thus can be used with the wild-type OE for *in vitro* transposition. A preferred way of practicing the present invention is to use a 54/242/372 mutant with an OE sequence that in combination provide a higher transposition activity than the 54/372 mutant and the OE sequence. Besides the combinations disclosed in Example 2 below, other "54/242/372 mutant-OE" combinations with transposition activity higher than the "54/372 mutant-OE"

combination can be readily identified by a skilled artisan using the method in Example 2 or other methods with which the skilled artisan is familiar.

[0022] In one aspect, the present invention relates to a Tn5 Tnp mutant or isolated Tn5 Tnp mutant that contains a mutation at position 54, 242, and 372 in comparison to the wild-type Tn5 Tnp and has greater avidity than wild-type Tn5 Tnp for at least one of the Tn5 OE sequence defined by SEQ ID NO:3 and the modified Tn5 OE sequence defined by SEQ ID NO:5. Preferably, the mutations are substitution mutations. Examples of mutations on a Tn5 Tnp mutant include but are not limited to glutamic acid to lysine or valine at position 54, leucine to proline or glutamine at position 372, and proline to alanine, glycine, valine, leucine, isoleucine, tyrosine, phenylalanine, or aspartic acid at position 242.

[0023] Optionally, a Tn5 Tnp mutant of the present invention further contains a mutation at position 56 in comparison to the wild-type Tn5 Tnp. For example, the methionine at position 56 of the wild-type Tn5 Tnp can be substituted with alanine. Although such a mutation has no apparent effect upon the transposase activity, it prevents translation of a Tn5 Tnp inhibitor protein encoded in partially overlapping sequence with the transposase, leading to a higher transposition rate. Weigand, T. W. and W. S. Reznikoff, "Characterization of Two Hypertransposing Tn5 Mutants," J. Bact. 174:1229-1239 (1992), incorporated herein by reference. Thus, a preferred Tn5 Tnp mutant of the present invention includes an amino acid other than methionine at amino acid position 56 to ensure the absence of the inhibitor from the *in vitro* system of the present invention (described below). However, it should be noted a position 56 mutation is not essential to the present invention because other means can be used to eliminate the inhibitor from the *in vitro* system. For example, the inhibitor protein can be separated from the Tn5 Tnp according to differences in size between the two proteins.

[0024] It is appreciated that additional amino acid sequences can be added to the N-terminus, C-terminus or both of a Tn5 Tnp mutant of the present invention without reducing the transposase activity to the level of the wild-type enzyme. A polypeptide or isolated polypeptide containing the Tn5 Tnp mutant flanked by the additional amino acid sequences as described above is within the scope of the present invention. A flanking amino acid sequence can but does not have to assist in purification, detection, or stabilization of the Tn5 Tnp mutant.

[0025] In another aspect, the present invention relates to a nucleic acid or isolated nucleic acid that contains a polynucleotide encoding a Tn5 Tnp mutant of the present invention. The nucleic acid can further contain a native or non-native transcription control sequence (e.g., a promoter)

operably linked to the Tn5 Tnp mutant-encoding polynucleotide. In addition, the present invention also encompasses a host cell that contains the nucleic acid of the present invention.

[0026] In another aspect, the present invention relates to a system for transposing a transposable DNA sequence *in vitro*. The system includes a polypeptide that contains a Tn5 Tnp mutant of the present invention, a donor DNA molecule containing the transposable DNA sequence that is flanked at its 5'- and 3'-ends by wild-type Tn5 OE sequences or modified Tn5 OE sequences that are active for *in vitro* transposition (defined below), and a target DNA molecule into which the transposable sequence can transpose.

[0027] In a related aspect, the present invention relates to a method of *in vitro* transposition using the transposition system described above. The method involves combining the donor DNA, the target DNA, and the Tn5 Tnp mutant-containing polypeptide in a suitable reaction buffer under suitable transposition conditions for a period of time sufficient for the transposase to catalyze the *in vitro* transposition. Details on suitable reaction buffers and reaction conditions are described in U.S. Patent No. 5,925,545 and Goryshin, I. Y., and Reznikoff, W. S., "Tn5 *in vitro* transposition," J. Biol. Chem. 273:7367-7374 (1998), incorporated by reference as if set forth herein in its entirety. Although in U.S. Patent No. 5,925,545 and Goryshin and Reznikoff (1998) the *in vitro* transposition was carried out with a two-step temperature incubation (below physiological temperature for binding of transposase to OE and physiological temperature for transposition), it is noted here that the whole procedure can also be carried out at a single temperature, the physiological temperature (e.g., 37°C).

[0028] The donor DNA can be circular or linear. If the donor DNA is linear, the OE sequences flanking the transposable DNA sequence can be at the termini of the linear donor DNA or the donor DNA can include some nucleotides upstream and downstream from the OE sequences.

[0029] Either wild-type or modified OE sequences can be used for flanking a transposable DNA sequence in the donor DNA molecule. Examples of modified OE sequences that confer an *in vitro* transposition frequency at least as high as the wild-type sequence are described in U.S. Patent No. 5,925,545. Other modified OE sequences not specifically described in U.S. Patent No. 5,925,545 can also be used as long as the combination of a sequence and a Tn5 Tnp mutant of the present invention results in a detectable level of transposition *in vitro*. Such modified OE sequences are referred to as modified OE sequences that are active for *in vitro* transposition and can be readily identified by a skilled artisan using the screening method disclosed in U.S. Patent No. 5,925,545.

[0030] The transposable DNA sequence between the OE sequences can include any desired nucleotide sequence. The length of the transposable DNA sequence between the OE sequences should be at least about 50 nucleotides, although smaller inserts may work. No upper limit to the insert size is known. However, it is known that a transposable DNA sequence of about 300 nucleotides in length can function well. By way of non-limiting examples, the transposable DNA sequence can include a coding region that encodes a detectable or selectable protein, with or without associated regulatory elements such as promoter, terminator, or the like.

[0031] If the transposable DNA sequence includes such a detectable or selectable coding region without a promoter, it will be possible to identify and map promoters in the target DNA that are uncovered by transposition of the coding region into a position downstream thereof, followed by analysis of the nucleic acid sequences upstream from the transposition site.

[0032] Likewise, the transposable DNA sequence can include a primer binding site that can be transposed into the target DNA, to facilitate sequencing methods or other methods that rely upon the use of primers distributed throughout the target genetic material. Similarly, the method can be used to introduce a desired restriction enzyme site or polylinker, or a site suitable for another type of recombination, such as a cre-lox, into the target.

[0033] The target DNA into which a transposable DNA sequence is transposed does not have any specific sequence requirements. Wild-type Tn5 Tnp has few, if any, preference for insertion sites. The Tn5 Tnp mutants disclosed here are believed to be the same. Accordingly, the method of the present invention can introduce changes into any target DNA.

[0034] In another aspect, the present invention relates to a method of using the Tn5 Tnp mutants disclosed herein for *in vitro* intra-molecular transpositions as described in U.S. Patent No. 5,948,622. The molecule involved in this method is a genetic construct that contains a transposable portion and a donor backbone portion. The transposable portion contains an origin of replication, a nucleotide sequence of interest, and a pair of the wild-type or modified Tn5 OE sequences. The method involves combining, in an *in vitro* reaction mix, a polypeptide that contains the Tn5 Tnp mutant with the genetic construct described above at a low concentration, to generate reaction products, transforming the reaction products into a host cell, proliferating the host cell to obtain multiple transformed cells, and selecting from among the multiple transformed cells for cells that contain a DNA molecule that has lost the donor backbone portion and that contain a transposition of the nucleotide sequence of interest. By low concentration, we mean that the genetic construct's concentration is relatively low so that intramolecular transposition, as opposed to intermolecular transposition, is encouraged. A skilled artisan can readily determine

the suitable low concentrations for a particular application. Generally speaking, the applicants have found a suitable amount of nucleic acid to be in the range of 0.05-0.005 μg/μl of reaction mix. At 0.05 μg/μl, 95% of the transposition events are intramolecular. At 0.005 μg/μl, or lower, about 100% of the events are intramolecular transpositions. Details on how to practice the method are described in U.S. Patent No. 5,948,622.

[0035] In another aspect, the present invention relates to a method for forming a synaptic complex *in vitro* between a Tn5 Tnp mutant herein disclosed and a polynucleotide that contains a transposable nucleotide sequence flanked by a pair of the wild-type or modified OE sequences. The method involves combining the Tn5 Tnp mutant with the polynucleotide *in vitro* under conditions that disfavor polynucleotide strand transfer. The synaptic complex formed can be introduced into a target cell under suitable conditions to make an insertional mutation at a random or quasi-random position in the cellular nucleic acid. By making an insertional mutation at a quasi-random position, we mean that the insertion event has a slight preference for one sequence over another. Details on how to form the synaptic complex and how to introduce the complex into a cell to make insertonal mutations are described in U.S. Patent 6,159,736.

[0036] The present invention will be more readily understood upon consideration of the following examples which are exemplary and are not intended to limit the scope of the invention.

Example 1

In Vivo Transposition with Tn5 Tnp Mutants

[0037] EK54/LP372/PA242 Tnp (proline to alanine mutation at position 242) and EK54/LP372/PG242 Tnp (proline to glycine mutation at position 242) were constructed by overlap PCR. Bases corresponding to aa 141-358 were amplified from pRZ10300 (Steiniger-White, M., and Reznikoff, W.S. "The C-terminal alpha helix of Tn5 transposase is required for synaptic complex formation," J. Biol. Chem. 275: 23127-33 (2000), incorporated by reference in its entirety) using Pfu polymerase and internal mismatched primers containing the mutation. The external primers included Tnp NheI and NotI sites. PCR products were digested with NheI (New England Biolabs, Beverly, MA) and NotI (Promega, Madison, WI) and ligated to the large NotI-NheI fragment of both pRZPET2 (Goryshin and Reznikoff, 1998) and pGRTYB35 (Bhasin, A., Goryshin, I.Y., Steiniger-White, M., York, D., and Reznikoff, W.S. "Characterization of a Tn5 pre-cleavage synaptic complex," J. Mol. Biol. 302: 49-63 (2000), incorporated by reference in its entirety). Each mutant Tnp was purified from its pGRTYB35 construct as described previously

(Bhasin et al., 1999). Because all mutations were created in an EK54/LP372 background, mutant Tnps will be defined by their additional mutations.

[0038] Mutations constructed in pRZPET2 were tested for *in vivo* activity using a papillation assay (Steiniger-White and Reznikoff, 2000). In this assay, the movement of a transposon having a promoterless *lacZ* gene flanked by OEs from its original plasmid to the chromosome is assessed. When the transposon inserts into the chromosome in the correct reading frame and orientation downstream from an active promoter and translation initiation signals, the *lacZ* gene is transcribed. Cells producing β-galactosidase have a growth advantage because they can utilize phenyl-β-D-galactoside provided in the media, while cells not producing β-galactosidase stop growing once all glucose is metabolized. Because cells producing β-galactosidase continue to grow, they will appear raised above the rest of the colony. These cells are made visible by including X-gal in the media. This assay allows qualitative assessment of *in vivo* transposition activity and showed that both EK54/LP372/PA242 Tnp and EK54/LP372/PG242 Tnp were hyperactive compared to EK54/LP372 Tnp.

Example 2

In Vitro Transposition with Tn5 Tnp Mutants

[0039] Eight Tn5 Tnp mutants were constructed by the method described in Example 1: EK54/LP372/PA242, EK54/LP372/PG242, EK54/LP372/PV242, EK54/LP372/PL242, EK54/LP372/PI242, EK54/LP372/PY242, EK54/LP372/PF242, and EK54/LP372/PD242. These mutants and EK54/LP372 Tnp were tested for *in vitro* transposition using two different substrates, pKJ1 and pKJ4. pKJ1 is a pUC19 vector with a 1,200 bp transposon (Tn) flanked by wild-type OEs (SEQ ID NO:3) while pKJ4 is isogenic to pKJ1 except that the Tn is flanked by modified OE sequence as defined by SEQ ID NO:5.

[0040] The *in vitro* transposition assay was performed as follows. 12 nM plasmid substrate (pKJ1 or pKJ4) was incubated with either 100 nM (for pKJ4) or 250 nM (for pKJ1) Tnp mutant in 100 mM potassium glutamate, 20 mM HEPES, pH 7.5, and 10 mM magnesium acetate at 37°C. 10 μL timepoints were taken at various intervals and added to 5 μL 1% SDS to stop the reaction. 5 μL of agarose gel loading dye were added following completion of all time points and 6 μL of each timepoint were run on a 1.3% agarose gel to separate reaction products. All reactions were performed in triplicate.

[0041] To determine the rate of decrease in substrate utilization for each Tnp mutant, the reaction products in each lane were quantitated using Total Lab software (Image Quant). The percentage of total DNA in each lane that corresponded to supercoiled substrate was then plotted versus time. These data were then fit to a one-phase exponential decay equation Y = If(X<X0, Plateau, Bottom + (Plateau - Bottom)*exp(-k*(X-X0))); where k is observed rate constant for the decrease in supercoiled substrate. Transposase activity was assessed as the rate of decrease in supercoiled substrate over time (k_{obs} (sec^{-1})).

[0042] As examples, the agarose gel pictures for mutants EK54/LP372/PA242 and EK54/LP372 are shown in Fig. 1A and Fig. 1B, respectively. The one-phase exponential curves for these two mutants are shown in Fig. 1C. The *in vitro* transposase activity (expressed as the rate of decrease in supercoiled substrate) of all the Tn5 Tnp mutants tested is summarized in Table 1. As shown in Table 1, all Tn5 Tnp mutants of the present invention tested had detectable *in vitro* transposition activity. When modified OE sequence defined by SEQ ID NO:5 (pKJ4) was employed for transposition, all eight Tn5 Tnp mutants of the present invention tested had higher activity than the EK54/LP372 mutant. When the wild-type OE sequence defined by SEQ ID NO:3 (pKJ1) was employed, five out of eight Tn5 Tnp mutants of the present invention tested had higher activity than the EK54/LP372 mutant.

Table 1. The observed rate constants for all Tn5 Tnp mutants tested on pKJ1 and pKJ4. Standard deviations are listed in parentheses.

	K_{obs} (sec^{-1}) for decrease in supercoiled substrate	
Tnp mutant	pKJ4 (with 100 nM Tnp)	pKJ1 (with 250 nM Tnp)
EK54/LP372	1.45 (0.08)	0.79 (0.03)
EK54/LP372/PA242	2.60 (0.09)	2.52 (0.14)
EK54/LP372/PG242	2.22 (0.08)	2.50 (0.10)
EK54/LP372/PV242	2.80 (0.13)	1.42 (0.06)
EK54/LP372/PL242	2.89 (0.19)	1.56 (0.07)
EK54/LP372/PI242	3.01 (0.36)	0.94 (0.03)
EK54/LP372/PY242	2.48 (0.13)	0.60 (0.04)
EK54/LP372/PF242	1.90 (0.12)	0.58 (0.03)
EK54/LP372/PD242	2.93 (0.18)	0.55 (0.03)

[0043] The foregoing examples are not intended to limit the scope of the invention. Rather the invention is understood to encompass all the variations and modifications that come within the scope of the appended claims.